A COMPARISON OF CREATININE MEASUREMENT BY THE JAFFE AND ENZYMATIC METHODS IN AN OUTPATIENT POPULATION

by

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STATEMENT OF THESIS APPROVAL

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ABSTRACT

Serum creatinine concentrations and estimated glomerular filtration rates (eGFR) are widely used for the evaluation of renal function. The Jaffe and enzymatic methods are the most common methods for creatinine measurement. The Jaffe method is commonly less expensive than enzymatic methods but may be more susceptible to interferences. Significant savings could be obtained if populations could be identified where the interference rate of the Jaffe method is acceptably low. The study objective was to compare creatinine and corresponding eGFR results from representative Jaffe and enzymatic creatinine methods in an outpatient population and determine the prevalence and magnitude of differences.

This study analyzed 543 unique, randomly selected, outpatient samples. Samples were analyzed using both the Jaffe and enzymatic creatinine methods using an Abbott Architect c8000. eGFRs were calculated using the CKD-EPI and MDRD equations.

Orthogonal (Deming) regression showed no significant difference between the two assays. The slope was 1.006 (95% CI: 0.998, 1.103) and the intercept was -0.005 (95% CI: -0.015, 0.006). The average difference (bias) was -0.007 mg/dL. The Bland-Altman limits of agreement (LOA) for the creatinine differences were -0.139 and 0.136 mg/dL. Of the CKD-EPI eGFR discrepancies, 3.1% resulted in a reclassification at the 60 mL/min/1.73m^2 decision limit. The difference between the predicted and actual CKD-EPI discordance at the 60 mL/min/1.73m^2 decision limit was not statistically significant (χ^2_2 = -0.13, p=0.89). Of the MDRD eGFR discrepancies, 4.8% resulted in a

reclassification at the 60 mL/min/1.73m^2 decision limit. The difference between the predicted and actual MDRD discordance at the 60 mL/min/1.73m^2 decision limit was not statistically significant ($\chi_2^2 = 0.31$, p=0.76).

Discrepancies in the CKD-EPI and MDRD eGFRs based on the Jaffe method did not result in a statistically significant increase in disease reclassifications at the 60 mL/min/1.73m^2 decision limit in an outpatient population. The number of discordant eGFR results slightly varied by what discordance criteria was used. An equal number of discordances were observed with the MDRD and CKD-EPI equations when eGFR were based upon measurement error (217 of 543). When discordance criteria was based upon observed differences (Bland-Altman), the MDRD equation showed slightly more discordances (26 of 543) compared to the CKD-EPI (21 of 543). Studies are needed to characterize the relative rate of interference in additional populations**.**

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INTRODUCTION

What is Creatinine?

Non-protein nitrogen (NPN) measurements have long been utilized to assess renal function as well as other clinical conditions. In the early part of the 20th century, NPN was used to describe those nitrogen containing compounds that remained in a filtrate after the proteins had been removed by precipitation. NPNs are now defined as nonprotein nitrogen containing compounds found in the blood.[1]

Creatinine is a NPN compound found in the blood and accounts for 1-2% of all NPN compounds. Creatinine is a waste product of creatine and phosphocreatine. Creatine is synthesized in the liver and pancreas, where it is released into the bloodstream and taken up by various tissues, particularly muscles and the brain. Within the tissues, the enzyme creatine kinase catalyzes the conversion of creatine to phosphocreatine, which acts as a form of energy storage. During activity, phosphocreatine converts to creatinine by removal of an inorganic phosphate. The creatinine is released into the bloodstream, where it is filtered and removed by glomerular filtration within the kidneys and released into the urine. The amount of creatinine formed daily is proportional to an individual's lean muscle mass and is released into circulation at a constant rate.[1]

Plasma creatinine concentration is the most widely used method for the evaluation of renal function. Plasma creatinine is used to diagnose and monitor acute and chronic renal disease, estimate glomerular filtration rate, and assess the status of renal dialysis

patients. Urine creatinine analysis is used to calculate creatinine clearance, confirm completeness of 24-hour urine collections, and serve as a reference quantity for other analytes, such as in the calculation of the albumin/creatinine ratio.[2-4] Several factors make creatinine a suitable compound for the evaluation of renal function: 1) plasma concentration is relatively constant , 2) it is completely cleared from the plasma at the glomeruli, 3) it is not reabsorbed by the tubules, and 4) it is convenient and inexpensive to measure.[1, 4] Limitations to creatinine measurements are variation due to age, gender, exercise, sample interferences, muscle mass, tubular secretion of creatinine causing an overestimation of creatinine clearance (7 to 10 percent increase), and individual diet may cause greater variation of creatinine excretion.[4]

Testing Methodology for Creatinine

The Jaffe and enzymatic methods are the most commonly used methods for measuring creatinine concentrations. Most routine creatinine assays in current use have evolved from the reaction first described by Jaffe in 1886.[5] The Jaffe method uses alkaline picrate to react with creatinine for analytical measurement. At an alkaline pH, creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The creatinine-picrate complex absorbs light at wavelengths near 500 nm. The rate of increase in absorbance is directly proportional to the concentration of creatinine in the sample.[3]

Although it is widely used, the Jaffe method is subject to nonspecificity bias. The nonspecificity bias occurs when the Jaffe reagent reacts with other substances within the matrix being tested. To account for the sensitivity of Jaffe methods to noncreatinine chromogens, some manufacturers have adjusted the calibration to minimize the non-specificity bias and produce results more closely aligned with reference methods.

Recalibration is performed by subtracting a constant value from the result to compensate for nonspecific chromogens. The constant value is a fixed concentration that is automatically subtracted from each result. The constant value is predetermined by the reagent manufacturer, which makes an assumption that noncreatinine chromogen interference is a constant between samples. Recalibrated Jaffe assays are referred to as compensated Jaffe methods. [5-7] Compensated assays can produce inaccurate results since this method is based on the assumption that the amount of nonspecific chromogens is constant among all samples.

Enzymatic methods are less likely to be affected by interferences than the Jaffe methods.[7] The enzymatic method used by Abbott Laboratories is based on a series of reactions. The first reaction is hydrolysis of creatinine to creatine. The creatine is then hydrolyzed by creatinase to sarcosine and urea. Sarcosine is oxidized by sarcosine oxidase to form glycine, formaldehyde, and hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and N-ethyl-N-sulphopropyl-m-toluidine (ESPMT) in the presence of peroxidase to create a color change. The resulting change in absorbance is measured at a wavelength of 548 nm and is proportional to the creatinine concentration of the sample.[2]

Interferences

Interfering substances are a significant issue in creatinine analysis. As previously mentioned, Jaffe methods are susceptible to nonspecificity bias from a large number of interfering substances. The enzymatic methods are less susceptible to interferences and provide a more reliable estimate of creatinine and glomerular filtration rate (GFR).[7-9]

Although the enzymatic method is more specific and less prone to endogenous interferences, interferences also occur in the enzymatic method. [5] The substances known to interfere with the enzymatic and Jaffe assay are listed in Table 1. Most likely, there are more interfering substances that have not yet been identified.

Clinical Use of Creatinine Assays

Creatinine levels are used to assess renal function and monitor chronic renal diseases. Although plasma creatinine values can provide useful data to clinicians, the values can be difficult to interpret because the serum creatinine concentration is affected by factors other than renal filtration. For this reason, equations have been developed to estimate the glomerular filtration rate (eGFR).[10-13]. These equations estimate the eGFR based on creatinine and adjust for factors such as age, sex, race, and body size.[12, 14] Common formulas used to estimate eGFR are the Cockcroft and Gault, the MDRD[12] (Modification of Diet in Renal Disease), and the CKD-EPI[14] (Chronic Kidney Disease Epidemiology Collaboration) formulas.

The University of Utah Hospital Clinical Laboratory (UHCL) uses the CKD-EPI equation to estimate eGFR. The CKD-EPI equation is:

$$eGFR_{CKD\text{-}EPI} = 141 \times \min(SC/k, 1)^{\alpha} \times \max(SC/k, 1)^{-1.209} \times 0.993^{Age} \times 1.018 \text{ (if female)} \times 1.159 \text{ (if black)} \quad \text{(Equation 1)}[14]$$

Where:

SC= Serum Creatinine

k is 0.7 for females and 0.9 for males

α is −0.329 for females and −0.411 for males

min indicates minimum of serum creatinine/k or 1

max indicates maximum of serum creatinine/k or 1.

eGFRCKD-EPI = (mL/min/1.73 m2)

The MDRD equation is:

eGFRMDRD =175 x (SCr-1.154) x (Age-0.203) x 0.742 (if female) x 1.212 (if black) (Equation 2)[12]

Where:

SCr= Serum Creatinine

eGFRMDRD = mL/min/1.73m2 [14-16]

The CKD-EPI and MDRD equations provide an estimate of the eGFR at single time points utilizing a patient's age, race, gender, and plasma creatinine concentration.[15, 16] eGFR estimates can be inaccurate if any of the input variables are inaccurate (age, ethnicity, and body mass index). Most importantly, eGFR estimates depend on the accuracy of creatinine measurements.[17-19]

The accuracy of creatinine measurements can have significant impact on the accuracy of eGFR estimates. eGFR is inversely related to creatinine (see equations 1 and 2). Because of the inverse relationship, the impact of imprecision and interfering substances is significant at low creatinine concentrations corresponding to eGFR values near the 60mL/min/1.73m^2 threshold for identifying kidney disease, but is less important at high creatinine-low eGFR values characteristic of severe kidney disease.[5] For example, suppose a 30-year-old Caucasian male has his blood collected for creatinine analysis and eGFR calculation. Under normal testing circumstances with no interfering

substances present, the patient's serum creatinine is 1.3 mg/dL, resulting in a CKD-EPI eGFR calculation of 73 mL/min/1.73 m^2. Due to the presence of a strong interfering substance, analysis of the sample yields a false high creatinine of 1.6 mg/dL. The falsely elevated creatinine value results in a eGFR of 57 mL/min/1.73 m^2. Chronic kidney diseases are classified using the Kidney Disease: Improving Global Outcomes (KDIGO) scale which is based on eGFR (Table 2). Using the correct eGFR (73 mL/min/1.73m^2), this patient would be classified as G2 which corresponds to normal to mild decrease in kidney function. In the presence of the interfering substance (GFR = 57 mL/min/1.73m^2), the patient would be classified as a G3a, which corresponds to moderate kidney disease.[15] Thus, an incorrect creatinine value can lead to misdiagnosis and improper treatment. If an interfering substance caused a patient's apparent eGFR to decrease below 60 mL/min/1.73 m^2 for three months or longer, the patient would be diagnosed as having chronic kidney disease.[10, 15, 20] Misclassification is a particular risk for patients whose eGFR values are 60 mL/min/1.73m^2, because diagnosis and treatment change at this point. Patients with creatinine values that are greatly elevated, resulting in a low eGFR, will see little change in the treatment and management of their disease (although continual assessment of eGFRs is important in determining the progress of CKD in these patients).[5]

UHCL Validation and Manufacturer Control Precision Study

Precision studies conducted during the UHCL Abbott Architect ci8200 validation (analyzers 1 and 2) were performed using Bio-Rad Multiqual 1 and 3 controls. Architect 1 precision for the Jaffe method at mean concentrations of 0.53 mg/dL and 6.96 mg/dL had total CVs of 1.9% and 0.8%, respectively. Architect 1 precision for the enzymatic

method at mean concentrations of 0.59 mg/dL and 6.97 mg/dL had total CVs of 3.6% and 2.0%, respectively. Architect 2 precision for the Jaffe method at mean concentrations of 0.53 mg/dL and 6.98 mg/dL had total CVs of 2.0% and 1.2%, respectively. Architect 2 precision for the enzymatic method at mean concentrations of 0.59 mg/dL and 7.02 mg/dL had total CVs of 4.3% and 0.78%, respectively. It should be noted that the number of runs for each method is different; Jaffe N=18, enzymatic N=27.[21]

In contrast, Abbott precision studies concluded that Jaffe methods have less precision than enzymatic methods. Precision for the Jaffe method at concentrations of 1.20 mg/dL and 4.66 mg/dL had total CVs of 4.95% and 3.18%, respectively.[3] Precision for the enzymatic method at concentrations of 0.65 mg/dL, 1.83 mg/dL, and 6.60 mg/dL had total CVs of 3.17%, 1.72%, and 0.95%, respectively.[2] Table 3 lists a summary of the precision comparisons for the UHCL Validation and the manufacturer's precision study.

Reagent Comparison Studies

Method comparison studies found in published literature using the Jaffe and enzymatic creatinine methods have produced varied results. One study compared nine different analyzers and found reasonably good agreement between the enzymatic and Jaffe creatinine methods; however, this study found that the Jaffe method lacked specificity (endogenous substances that interfered with the reaction) and concluded that an alternative method may be needed in certain clinical situations (see Table 1 for common interferences).[5] Method comparisons on patient groups with specific diagnoses found that values obtained using Jaffe methods were less than corresponding values using enzymatic methods in liver disorder patients (Jaffe mean difference: -8.9%; Spearman's rho= -0.378, $p < 0.001$; CV not available)[6] and diabetics with ketoacidosis (Jaffe mean

difference: +50%, CV not available)[22]. A large study involving 144 participating laboratories examined 11 pairs of value assigned creatinine specimens ranging from 0.59-2.96 mg/dL using Jaffe or enzymatic methodologies. The study found that Jaffe methods overestimated creatinine concentrations at 21%, 12% and 10% for target values of 0.59, 0.83, and 1.06 mg/dL, respectively. Enzymatic methods obtained values that estimated differences at 0%, -1%, and -2%, respectively.[23] These studies provide evidence that the Jaffe method is less accurate and the Jaffe method is affected by a wider range of interferences than the enzymatic method.

Reagent Cost Comparison

Cost is an important consideration in the selection of a laboratory method. Abbott list prices for the reagents are $580 for 1,875 creatinine Jaffe tests ($0.31 per test) and $1,509 for 750 enzymatic tests ($2.01 per test).[24] The testing volume of creatinine at UHCL is approximately 190,000 tests per year (Note: This does not include testing performed at Huntsman Cancer Hospital Laboratory or South Jordan Hospital Laboratory.) Costs based on yearly tests performed are $58,900 for Jaffe reagent and $381,900 for enzymatic. The potential cost savings of performing creatinine testing with Jaffe reagent is an estimated $323,000 per year. Cost savings may vary based upon reagent manufacturer.

Method Risk

Risk is the product of two factors: 1) the probability of an event, and 2) the consequence associated with the event.[25] The risk associated with the Jaffe method could be acceptable if either the probability of interference or the clinical consequences

associated with interferences were sufficiently low. Unfortunately, neither of these risk components has been characterized for creatinine assays. Both risk components could vary by patient population. Therefore, it may be possible to find patient populations for which the probability of interference or the consequence of inaccurate results is low.

An interference will only have consequences if the observed change in measurement is considered both statistically and clinically significant. To be classified as statistically significant, the magnitude of the change must be greater than the normal background variation. To be "clinically significant," a change in measurement must result in a change in management (investigation, treatment, or monitoring) or patient outcome.

Method Preference

Laboratory methods should be selected carefully, considering both costs and patient care. The choice between the Jaffe and enzymatic methods present a tradeoff between cost and accuracy. The enzymatic method is more costly but is also more accurate.

It may be possible to find situations in which the Jaffe method provides acceptable performance. The Jaffe could be acceptable in contexts where the probability of inaccurate results is low or if the consequences of inaccurate results could be minimized. For example, laboratories might be able to identify patient populations with different risk of interference and create separate testing panels for each population. It could be suggested that populations that are found to have a high prevalence of interfering substances have the option to order a different testing panel (Example: basic metabolic panel (BMP), comprehensive metabolic panel (CMP), etc.) that utilizes

enzymatic methodology. A second testing panel that utilizes the cheaper Jaffe methodology would be available to populations that are found to have a low prevalence of interfering substances. Using a structured ordering process based on prevalence of interference would give a laboratory the opportunity to save money using reliable testing with enzymatic methodology for at-risk patient populations.

Study Objective

The purpose of this study was to compare the creatinine and eGFR results from Jaffe and enzymatic creatinine methodologies in the University of Utah Healthcare outpatient clinic populations to analyze the prevalence of discordant results due to method interference and associated risk of implementing the Jaffe method. Many studies have reported on differences between the Jaffe and enzymatic technique.[26, 27] Most studies have looked at discordances in creatinine values or estimated glomerular filtration rates using samples spiked with an interfering substance. To our knowledge, no studies have used a risk evaluation framework to evaluate the prevalence of interferences in specific patient populations to identify any groups which are deemed unsafe to use the creatinine Jaffe method.

Table 1: List of Common Creatinine Interferences

Interferences				
Compound	**Exposure Population, Prevalence**	**Impact on Jaffe (Direction, Magnitude)**	**Impact on Enzymatic (Direction, Magnitude)**	**References**
5-aminolevulinic acid	Photodynamic Therapy Patients, Common	Increased, Major	None	[32]
Ascorbic Acid	Any, Common	Increased, Minor	Decreased, Minor	[5, 9, 33]
Albumin, High	Any, Common	Increased, Minor	None	[5, 7, 34]
Bilirubin, High	Liver Disorder Patients, Uncommon	Decreased, Minor-Major (magnitude increases with higher levels)	Decreased, Minor	[5, 7, 9, 33, 34]
Catecholamines (Therapeutic)	Patients Receiving Catecholamines, Uncommon	None	Decreased, Major	[5, 9, 33, 35]
Cephalosporins	Any, Common	Increased, Minor-Major	None	[5, 9, 33]
Creatine	Any, Common	Increased, Minor	None	[5, 9]
Glucose, High	Diabetic Patients, Uncommon	Increased, Minor	None	[5, 7, 9, 33]
Hemoglobin F	Neonates, Common	Decreased, Major	None	[5, 7, 9, 34]
Homogentisic Acid	Alkaptonuric Patients (Urine), Rare	None	Decreased, Major	[36]
IgG, High		Increased, Minor	None	[34]
Ketones	Diabetic Patients, Uncommon	Increased, Minor-Moderate	None	[5, 9, 22, 33]
Lidocaine	Cardiac Patients (Ventricular Arrhythmias, Acute MI, Cardiac Catheterization), Uncommon	Increased, Minor	Increased, Moderate	[33]
Nitromethane	Any, Rare	Increased, Major	None	[37, 38]
Phenolsulfonphthalein (Urine)	Patients Undergoing Kidney Function Analysis, Rare	Increased, None-Moderate	Unknown	[39]
Sulfobromophthalein	Patients Undergoing Liver Function Analysis, Rare	Increased, None-Moderate	Unknown	[39]
Total Protein, High	Any, Uncommon	Increased, Minor	None	[33]

*Note that there are many unknown potential interferences for these assays. Table 1 lists common interferences found in the literature and is not a comprehensive list, as many interferences are still unknown.

Table 2: Kidney Disease: Improving Global Outcomes (KDIGO) Classifications of Chronic Kidney Disease Based on eGFR[40]

KDIGO Classification of CKD		
Glomerular Filtration Rate (GFR)		
Category	**GFR**	**Degree of Renal Function**
G1	≥90	Normal or High
G2	60-89	Mild Decrease
G3a	45-59	Mild to Moderate Decrease
G3b	30-44	Mild to Severe Decrease
G4	15-29	Severe Decrease
G5	<15	Kidney Failure

Table 3: UHCL Validation and Abbott Diagnostics Precision data

	Total CV Jaffe (N=18)		Total CV Enzymatic (N=27)		
	Control 1 0.53 mg/dL	**Control 2 7.00 mg/dL**	**Control 1 0.59 mg/dL**	**Control 2 7.00 mg/dL**	
Architect 1 (UHCL Validation)	1.90%	0.80%	3.62%	2.00%	
Architect 2 (UHCL Validation)	2.00%	1.20%	4.33%	0.78%	
	Total CV Jaffe (N=80)		**Total CV Enzymatic (N=80)**		
	1.20 mg/dL	**4.66 mg/dL**	**0.65 mg/dL**	**1.83 mg/dL**	**6.60 mg/dL**
Abbott Precision (Package Insert)	4.95%	3.18%	3.17%	1.72%	0.95%
"N=" indicates number of runs performed					

Architect 1 and 2 data are precision data from the UHCL reagent validation. Abbott Precision is the precision data obtained from the reagent package inserts summarizing precision data performed by Abbott Diagnostics.

MATERIALS AND METHODS

Literature Review

The literature review was performed in two phases. The first phase consisted of searching for appropriate research articles using keyword searches in PubMed and Cumulative Index to Nursing and Allied Health (CINAHL). The following are the search terms that were used: *enzymatic creatinine, enzymatic creatinine interferences, Jaffe creatinine, Jaffe creatinine interferences, creatinine interferences, enzymatic vs Jaffe, glomerular filtration rate, kidney function tests, CKD-EPI, MDRD,* and *compensated Jaffe*. Additional references that were obtained outside of the keyword searches were the Abbott Jaffe and enzymatic reagent package inserts, UHCL Abbott Architect Validation data, Clinical Chemistry: Concepts and Applications, and Effects of Disease on Clinical Laboratory Tests: 4th Edition. Citations were entered into EndNote version 6.

In the second phase, the research articles selected during the first phase were evaluated using Scopus to perform citation (forward) and reference (backward) searches. Duplicate references were removed using Endnote's duplicate removal function followed by a manual search.

Patient Population

This study was based on samples collected from patients attending outpatient clinics both at the University Hospital and surrounding community clinics. All inpatient, cancer, emergency department, and surgical locations/clinics were excluded from this

study. The use of patient samples was approved by the University of Utah Institutional Review Board (IRB 00065210).

Sample Collection

Clinic samples are typically collected and received in the lab during weekdays only (Monday through Friday). Samples were randomly selected from the daily volume of incoming samples. The ARUP Information Technology department developed a report that listed all creatinine assays performed on samples originating from hospital and community outpatient clinics from the previous day. The report included the sample accession, clinic name, and patient information (medical record number (MRN), age, sex, and race). Samples were randomly selected from this list using a random number generator in Microsoft Excel. Each sample was assigned a random number between 0.0000 to 1.0000 (four decimal places were used). The samples were then ordered according to the random number and the required number of samples was obtained by selecting samples in ascending order from the ordered list. The MRNs of selected samples were compared against a list of previously selected samples. Samples drawn from patients that were previously sampled were excluded to ensure that each sample was from a unique patient.

Samples were selected in two phases. In the first phase, 200 samples were collected at a rate of 20 per day. In the second phase, 343 samples were collected at a rate of 50 samples per day (using the same random sample selection process that was used in the first phase). All samples were retrieved from the UHCL's refrigerated storage ($4^{o}C$) and recentrifuged prior to analysis. Samples were analyzed one day after the samples were collected (testing occurred Tuesday through Saturday).

Overall, we selected 543 unique outpatient samples over a period of 45 days (7/31/2013 to 9/13/2013). We obtained 248 samples from outpatient clinics located at the University of Utah Hospital and 295 samples from outpatient clinics located outside the hospital.

Sample Analysis

Sample analysis was performed on the Abbott Architect c8000 analyzer. Each sample was tested by both Jaffe (kinetic alkaline picrate, Abbott Laboratories; Abbott Park, IL) and enzymatic (creatininase, Abbott Laboratories; Abbott Park, IL) methods. After centrifugation, samples were loaded onto Architect sample trays and batch ordered to perform simultaneous Jaffe and enzymatic testing.

Two levels of quality control (QC) were analyzed daily before any sample testing was performed. QC materials used for both methodologies were Bio-Rad MultiQual levels 1 and 3. For the Jaffe method, calibrations were performed every five days, as directed by the package insert, using Abbott MCC (Multi-constituent Calibrator). For the enzymatic method, calibrations were performed every 60 days, as directed by the package insert, using Abbott CCC (Clinical Chemistry Calibrator).

Reagents

Abbott Jaffe Creatinine utilizes two reagents. Reagent 1 is sodium hydroxide and Reagent 2 is picric acid. The assay reaction occurs at an alkaline pH. The creatinine in the sample reacts with picrate to form a creatinine-picrate complex. The rate of increase in absorbance at 500nm due to the formation of this complex is directly proportional to the concentration of creatinine in the sample.[3]

Abbott Enzymatic Creatinine utilizes two reagents. Reagent 1 contains Good's buffer (pH 7.5), creatininase, sarcosine oxidase, catalase, and ESPMT. Reagent 2 contains Good's buffer (pH 7.5), creatininase, peroxidase, 4-aminoantipyrine. The assay reaction occurs when the creatinine is hydrolyzed by creatininase to creatine. Creatine is in turn hydrolyzed by creatinase to sarcosine and urea. Sarcosine is oxidized by sarcosine oxidase to glycine and formaldehyde, with the concomitant production of hydrogen peroxide. The hydrogen peroxide reacts with 4-aminoantipyrine and ESPMT in the presence of peroxidase to yield a quinoneimine dye. The absorbance change at 548 nm is proportional to the creatinine concentration in the sample.[2]

Creatinine Precision Measurement

A 20 day precision study was performed using the Abbott Creatinine Jaffe and enzymatic assays on the Abbott Architect c8000. This precision study was performed to determine the precision of both reagents that only includes short-term sources of within-run and between-run variation by eliminating variation due to different reagent lots, calibrations (enzymatic only), instruments, and operators.

A "What If" analysis was performed within Excel using the community clinic Jaffe eGFR results to find an average creatinine concentration at a GFR of 60 mL/min/1.73m^2 (creatinine= 1.20 mg/dL). The "What If" analysis is an Excel tool that was used to evaluate what the creatinine concentrations for a given patient would be if the patients eGFR was changed to a specific value. This tool allowed for a reverse calculation for average creatinine concentration (and includes factors such as age, race and gender) to be determined by changing the eGFR calculation to solve for creatinine concentration in place of eGFR.

The 20 day precision study was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines and evaluated five different concentration pools of creatinine. Concentration pools were selected to include the range of clinic sample concentrations that were observed in the community clinic study (excluding outlier creatinine results greater than 5.0 mg/dL), and at points that were determined to have clinical significance (0.84 mg/dL and 1.20 mg/dL). Each patient pool was created using a minimum of 10 separate samples that were close to the desired concentration. The lowest concentration pool was diluted with saline to obtain the desired concentration.

Creatinine sample concentration pools were made at 0.28 mg/dL, 0.79 mg/dL, 1.21 mg/dL, 2.73 mg/dL, and 5.08 mg/dL. Each concentration pool was separated into 20 vials and frozen at -30^{o}C. One sample at each concentration was measured in duplicate, daily, for 20 consecutive days. The total standard deviation (within run and between run) was determined from the 20 specimens at each concentration.

eGFR precision was determined by simulation using the 20-day creatinine precision data. Measurement errors in serum creatinine were assumed to be normally distributed. The variation in eGFR was determined by taking random samples from the creatinine distribution and calculating the corresponding eGFRs. The standard deviation for eGFR for each pooled sample was determined from 10,000 simulated iterations. Standard deviations were determined for the eGFR predicted by the MDRD and CKD-EPI methods using creatinine measured by the Jaffe and enzymatic methods.

Statistical Analysis

Creatinine results for both testing methodologies were compiled into an Excel spreadsheet that included sample accession, clinic name/location, MRN, age, sex, and

race. Calculations were performed using Stata 12 (Stata Corporation, College Station, TX). Statistical hypotheses were tested at the 5% significance level. Correlation analysis was performed using orthogonal (Deming) regression using Minitab 16 (Minitab Inc., State College, PA). The observed and expected discordance rate was determined for eGFR measurements at the 60 mL/min/1.73m^2 decision limit.

Analysis of Discordant Creatinine Results

Discordant creatinine measurements were identified by comparing the observed difference (Jaffe – enzymatic) to a measure of variation. We used two different measures of variation to determine discordance. The first method was based on the observed variation of differences. The second method was based on the predicted variation due to imprecision in measurement (determined by 20 day precision study).

Discordance based on observed variation was standardized using the standard deviation of the observed differences:

$$z_{C,JE}^{obs} = \frac{C_J - C_E}{S_{C,JE}^{obs}} \qquad \text{(Equation 3)}$$

where $z_{C,JE}^{obs}$ is the standardized difference in creatinine values obtained by the Jaffe and enzymatic methods, C_J is the creatinine concentration obtained by the Jaffe method, C_E is the creatinine concentration obtained by the enzymatic method, and $S_{C,JE}^{obs}$ is the standard deviation based on the observed differences, $(C_J - C_E)$ and includes all sources of error (measurement error, interferences, etc.). Cases with $|z_{C,JE}^{obs}| > 1.96$ were defined as discordant. By, definition, this criterion will classify 5% of cases as discordant. Using this definition, discordant results could be caused by imprecision or interference.

Discordance based on measurement variation was standardized using the predicted standard deviation of the differences due to measurement imprecision:

$$z_{C,JE}^{Prec} = \frac{C_J - C_E}{S_{C,JE}^{Prec}} \quad \text{(Equation 4)}$$

where $z_{C,JE}^{Prec}$ is the standardized difference in creatinine values obtained by the Jaffe and enzymatic methods, $S_{C,JE}^{Prec}(C_E) = \sqrt{\left[s_{C,J}^{Prec}(C_E)\right]^2 + \left[s_{C,E}^{Prec}(C_E)\right]^2}$ is the standard deviation of the creatinine difference due to measurement imprecision. $s_{C,J}^{Prec}(C_E)$ and $s_{C,E}^{Prec}(C_E)$ are the total measurement variance (between day + between run + between sample) associated with the Jaffe and enzymatic methods, respectively, at a particular creatinine level as determined by the reference (enzymatic) method. Cases with $|z_{C,JE}^{Prec}| > 1.96$ were defined as discordant. $\left[s_{C,J}^{Prec}(C_E)\right]^2$ and $\left[s_{C,E}^{Prec}(C_E)\right]^2$ were obtained by linear interpolation of the standard deviation data obtained from the precision studies. $S_{C,JE}^{Prec}(C_E)$ represents the predicted imprecision due to measurement error alone and does not include the impact of other factors such as interferences. When interferences are absent, this definition would define 5% of cases as discordant. When interferences are present, this definition would classify more than 5% of cases as discordant. By definition, interferences cause measurement errors that are greater than the measurement imprecision. Thus, by using a definition of discordance based on imprecision, the incremental gain in discordances can be attributed to interference.

Analysis of Discordant eGFR Results

As with creatinine measurements, we identified eGFR discordances using both predicted measurement error and the distribution of observed differences. Discordant eGFRs based on observed differences were determined using the MDRD and CKD-EPI equations:

$$z^{obs}_{G(M),JE} = \frac{G_{J,M} - G_{E,M}}{S^{obs}_{G(M),JE}} \qquad \text{(Equation 5)}$$

where $z^{obs}_{G(M),JE}$ is the standardized difference in eGFR obtained by the Jaffe and enzymatic methods using method M (M=MDRD or CKD-EPI), $G_{J,M}$ is the eGFR concentration obtained by the Jaffe method using eGFR method M, $G_{E,M}$ is the eGFR obtained by the enzymatic method, and $S^{obs}_{G(M),JE}$ is the standard deviation based on the observed differences, $(G_{J,M} - G_{E,M})$.

Discordant eGFRs based on imprecision between the Jaffe and enzymatic methods were standardized using the standard deviation of the difference due to measurement:

$$z^{Prec}_{G(M),JE} = \frac{C_J - C_E}{S^{Prec}_{G(M),JE}} \qquad \text{(Equation 6)}$$

$$S^{Prec}_{G(M),JE}(C_E) = \sqrt{\left[s^{Prec}_{G(M),J}(C_E)\right]^2 + \left[s^{Prec}_{G(M),E}(C_E)\right]^2} \qquad \text{(Equation 7)}$$

where $z^{Prec}_{GM,JE}$ is the standardized difference in eGFR obtained by the Jaffe and enzymatic methods, $S^{Prec}_{G(M),JE}(C_E)$ is the standard deviation of eGFR difference due to measurement, and $s^{Prec}_{C,J}$ and $s^{Prec}_{C,E}$ are the total measurement variance (between day + between run +

between sample) associated with the Jaffe and enzymatic methods, respectively. Cases with $z^{Prec}_{G(M),JE} > 1.96$ were defined as discordant. $s^{Prec}_{G(M),J}(C_E)$ and $s^{Prec}_{G(M),E}(C_E)$ were obtained by simulation as described above. $s^{Prec}_{G(M),J}(C_E)$ and $s^{Prec}_{G(M),E}(C_E)$ are the predicted imprecision in eGFR due to measurement error alone and do not include the impact of other factors such as interferences and operator error.

Expected Discordance Rate

The expected discordance rate was measured as follows:

Let:

x = the true underlying value for the analyte

X_M= the observed value using method M, including all sources of error

X_M^{Prec}= the observed value using method M, including measurement error only

$b_M(x)$ = the bias in X_M^{Prec} as a function of x

$s_M(x)$ = the precision profile of method M (standard deviation of X_M^{Prec}as a function of x)

c = the diagnostic cutoff

$z_M(x) = \frac{x+b_M(x)-c}{s_M(x)}$ = the standardized distance of the mean of method M from the cutoff

$F_M(z|x)$ = the cumulative distribution function for the measurement error for laboratory method M, given the true value of the underlying analyte x

$\Phi(z)$ = the normal cumulative distribution function

$g(x)$ = the density function for the concentration of the analyte in the patient population

Let $A_{A,B}$ be the event that the two laboratory methods agree with respect to the diagnostic cutoff. Given two observations on the same sample, there are two ways that the results of the methods can agree (see Figure 1):

$$A_{A,B} = \begin{cases} 1 \; if \; [X_A < c) \text{ AND } (X_B < c)] \text{ OR } [(X_A > c) \text{ AND } (X_B > c)] \\ 0 \; otherwise \end{cases}$$

(Equation 8)

The agreement rate is:

$$\bar{A}_{A,B}^{obs} = P\left(A_{A,B}\right) \qquad \text{(Equation 9)}$$

If imprecision is the only source of variation, then:

$$A_{A,B}^{Prec} = \begin{cases} 1 \; if \; [X_A^{Prec} < c) \text{ AND } (X_B^{Prec} < c)] \text{ OR } [(X_A^{Prec} > c) \text{ AND } (X_B^{Prec} > c)] \\ 0 \; otherwise \end{cases}$$

(Equation 10)

Given a true value, x, the probability of agreement between two observations with respect to the cutoff is:

$$P\left(A_{A,B}^{Prec}|x\right) = P\{[(X_A^{Prec} < c) \text{ AND } (X_B^{Prec} < c)] \text{ OR } [(X_A^{Prec} > c) \text{ AND } (X_B^{Prec} > c)]|x\}$$

(Equation 11)

The expected agreement rate with respect to the cutoff is:

$$\bar{A}_{A,B}^{Prec} = P\left(A_{A,B}^{Prec}\right) = \int_0^\infty \mathrm{P}\left(A_{A,B}^{Prec}|x\right) g(x)dx$$

$$= \int_0^\infty \{F_A(c|x)F_B(c|x) + [1 - F_A(c|x)][1 - F_B(c|x)]\} g(x)dx$$

$$= \int_0^\infty \left\{\Phi(-z_A(x))\Phi\left(-z_B(x)\right) + [1 - \Phi(-z_A(x)][1 -\right.$$

$$\Phi(-z_B(x)]\,\}g(x)dx$$

(Equation 12)

where the last step is based on the assumption that the measurement error X_M^{Prec} is normally distributed with mean $b_M(x)$ and standard deviation $s_M(x)$ when the true value of the analyte is x.

RESULTS

20 Day Creatinine Precision Profile

The precision profiles are presented in Table 4. The Jaffe method had greater precision than the enzymatic method, except at the lowest concentration level (0.28 mg/dL). The standard deviation of the difference between the Jaffe and enzymatic methods ranged from 0.012 mg/dL (SCr = 0.278 mg/dL) to 0.71 mg/dL (SCr = 5.080 mg/dL).

Correlation of Creatinine Measurements

Orthogonal (Deming) regression analysis showed no significant difference between the Jaffe and enzymatic methods (Figure 1). The slope was 1.006 (95% CI: 0.998, 1.103) and the intercept was -0.005 (95% CI: -0.015, 0.006).

Discordant Creatinine Measurements

The distribution of differences in creatinine measurements between the Jaffe and enzymatic method are shown in Figure 2. The average difference (bias) between the methods was -0.007 mg/dL. Forty percent of the differences exceeded the bounds (two standard deviations) predicted by measurement error alone (Figure 1). These were classified as discordant results. There was no association between discordant results and clinic location ($\chi_2^2 = 1.3$, p=0.53). Five percent of the differences exceeded the bounds

based on observed differences. There was no association between this set of outliers and outpatient clinic type ($\chi_2^2 = 0.02$, p=0.89).

eGFR Precision Profile

The precision profiles for the eGFR are presented in Figure 3. When using the CKD-EPI method, the predicted standard deviation for the difference in eGFR (Jaffe vs. enzymatic) ranged from 0.1 mL/min/1.73m^2 (CV= 1.25%) to 3.5 mL/min/1.73m^2 (CV= 2.78%), at eGFR levels of 8 and 126 mL/min/1.73m^2, respectively. The precision profile for CKD-EPI was discontinuous when the eGFR was greater than 80 mL/min/1.73m^2 because of the minimum and maximum functions in the estimating equation. When using the MDRD method, the predicted standard deviation for the difference in eGFR (Jaffe vs. enzymatic) ranged from 0.1 mL/min/1.73m^2 (CV= 1.25%) to 5.8 mL/min/1.73m^2 (CV= 3.28%) at eGFR levels of 8 and 177 mL/min/1.73m^2, respectively.

Correlation of eGFR Measurements

Orthogonal (Deming) regression showed significant differences between eGFRs based on the Jaffe and enzymatic methods (Figure 4). For the CKD-EPI method, the y-intercept for the Jaffe-enzymatic correlation was 2.66 (95% CI: 1.35, 3.98) and the slope was 0.97 (95% CI: 0.95, 0.99). For the MDRD method, the y-intercept was 6.49 (95% CI: 4.8, 8.2) and the slope was 0.90 (95% CI: 0.88, 0.92).

Discordant eGFR Measurements

Discordant results for the CKD-EPI method are summarized in Table 5. Forty percent (217 of 543) of the differences in eGFR (Jaffe - enzymatic) exceeded two standard deviations of the predicted measurement error. With the CKD-EPI equation,

3.9% of the differences (21 of 543) exceeded the Bland-Altman limits of agreement (Figure 5). At the 60 mL/min/1.73m^2 decision limit, 3.1% (17 of 543) of differences resulted in a change of classification. Of these, six were within the expected measurement variation and 11 exceeded two standard deviations of the difference based on measurement error. The predicted discordance rate at the 60 mL/min/1.73m^2 decision limit was 3.3% (17.7 of 543). The difference between the predicted and actual discordance at the 60 mL/min/1.73m^2 decision limit was not statistically significant (χ_2^2 = -0.13, p=0.89).

Discordant results for the MDRD method are summarized in Table 6. Forty percent (217 of 543) of the differences in eGFR (Jaffe – enzymatic) exceeded two standard deviations of the predicted measurement error of the difference. With the MDRD equation, 4.8% of the differences (26 of 543) exceeded the Bland-Altman limits of agreement (Figure 5). At the 60 mL/min/1.73m^2 decision limit, 4.8% (26 of 543) of the observed eGFR differences resulted in a change in classification. Of these, nine were within the expected measurement variation and 17 exceeded two standard deviations of the difference based on measurement error. The predicted discordance rate at the 60 mL/min/1.73m^2 decision limit was 4.4% (24.2 of 543). The difference between the predicted and actual discordance at the 60 mL/min/1.73m^2 decision limit was not statistically significant (χ_2^2 = 0.31, p=0.76). The MDRD discordant differences were highly correlated with CKD-EPI discordant differences (ρ = 0.97, p =<0.001)

Expected vs. Actual Discordance Rate

The expected discordance rates were calculated for both eGFR equations using Equation 12 and were calculated at decision limits of 30 mL/min/1.73m^2,

45mL/min/1.73m^2, and 60mL/min/1.73m^2 and are summarized in Tables 7 and 8. For the CKD-EPI equation, the observed discordance rate was 0.37, 1.47 and 3.13, respectively. The expected discordance rate from imprecision (enzymatic only) was 0.28, 0.82, and 1.46, respectively. The estimated discordance rate due to factors other than imprecision was 0.02 , 0.62, and 1.72, respectively.

For the MDRD equation, the observed discordance rate was 0.37, 0.74, and 4.79, respectively. The expected discordance rate from imprecision (enzymatic only) was 0.26, 0.78, and 1.66, respectively. The estimated discordance rate due to factors other than imprecision was 0.12, -0.01, and 3.23, respectively.

Table 4: Precision Profile for Creatinine Assays

Creatinine Level (mg/dL)			Standard Deviation		CV (%)	
Enzymatic	Jaffe	Absolute Value of the Difference	Enzymatic	Jaffe	Enzymatic	Jaffe
0.278	0.288	0.012	0.008	0.009	2.91	3.00
0.791	0.772	0.018	0.014	0.011	1.71	1.43
1.214	1.126	0.029	0.021	0.020	1.71	0.81
2.734	2.822	0.042	0.035	0.023	1.27	0.81
5.080	5.128	0.071	0.059	0.040	1.16	0.78

Table 5: Cross-Tabulation of Discordant eGFR Results using CKD-EPI

Discordance Type	Discordant Result	Decision Limit		Total
		60 mL/min/ 1.73m^2		
		Discordant		
		No	Yes	
Based on Measurement Error	No	320	6	326
	Yes	206	11	217
	Total	526	17	543
Based on Observed Differences (Bland-Altman)	No	505	17	522
	Yes	21	0	21
	Total	526	17	543

Discordances are classified by discordance type and with respect to reclassification at decision limits. Discordance based on measurement error is determined relative to limits calculated by Equation 7. Discordance based on observed differences is determined relative to limits calculated by Equation 5.

Table 6: Cross-Tabulation of eGFR Discordant Results using MDRD

Discordance Type	Discordant Result	Decision Limit		Total
		60 mL/min/1.73m^2		
		Discordant		
		No	Yes	
Based on Measurement Error	No	317	9	326
	Yes	200	17	217
	Total	517	26	543
Based on Observed Differences (Bland-Altman)	No	491	26	517
	Yes	26	0	26
	Total	517	26	543

Discordances are classified by discordance type and with respect to reclassification at decision limits. Discordance based on measurement error is determined relative to limits calculated by Equation 7. Discordance based on observed differences is determined relative to limits calculated by Equation 5.

Table 7: Expected vs. Actual Discordance Rate using CKD-EPI

		CKD-EPI		
		Decision Limit, mL/min/1.73m^2		
		30	45	60
A	**Observed discordance rate**	0.37	1.47	3.13
B	**Expected discordance rate from enzymatic alone (imprecision)***	0.28 0.06-0.53	0.82 0.54-1.13	1.46 1.01 – 2.02
C	**Expected discordance rate for enzymatic and Jaffe (imprecision only)**	0.35 0.08-0.56	0.85 0.59-1.22	1.41 0.95-1.95
D	**Incremental discordance rate from Jaffe (A-B)**	0.09 -0.16-0.31	0.65 0.34-0.93	1.67 1.11-2.12
E	**Estimated discordance rate due to factors other than imprecision (A-C)**	0.02 -0.19-0.29	0.62 0.35-0.88	1.72 1.18-2.18

* = discordance rate we would observe if we compared enzymatic against enzymatic

Table 8: Expected vs. Actual Discordance Rate using MDRD

		MDRD		
		Decision Limit, mL/min/1.73m^2		
		30	**45**	**60**
A	**Observed discordance rate**	0.37	0.74	4.79
B	**Expected discordance rate from enzymatic alone (imprecision)***	0.26 0.04-0.51	0.78 0.50-1.12	1.66 1.21-2.24
C	**Expected discordance rate for enzymatic and Jaffe (imprecision only)**	0.25 0.04-0.49	0.75 0.49-1.09	1.56 1.00-2.12
D	**Incremental discordance rate from Jaffe (A-B)**	0.11 -0.12-0.33	-0.04 -0.38-0.24	3.13 2.55-3.58
E	**Estimated discordance rate due to factors other than imprecision (A-C)**	0.12 -0.12-0.33	-0.01 -0.45-0.25	3.23 2.67-3.79

* = discordance rate we would observe if we compared enzymatic against enzymatic

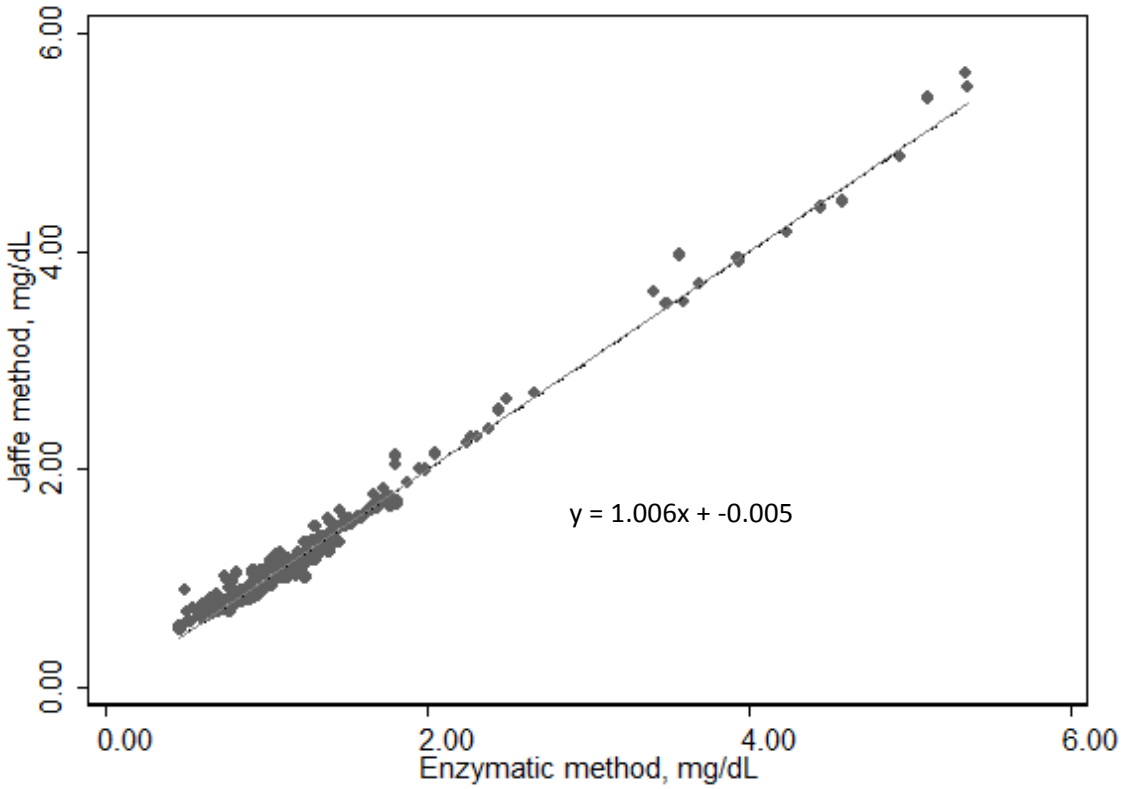

Figure 1: Correlation of Jaffe and enzymatic methods. The solid line indicates perfect concordance and the dashed line is the best linear fit comparing Jaffe and enzymatic creatinine values. Lines observed represent perfect concordance. n=543

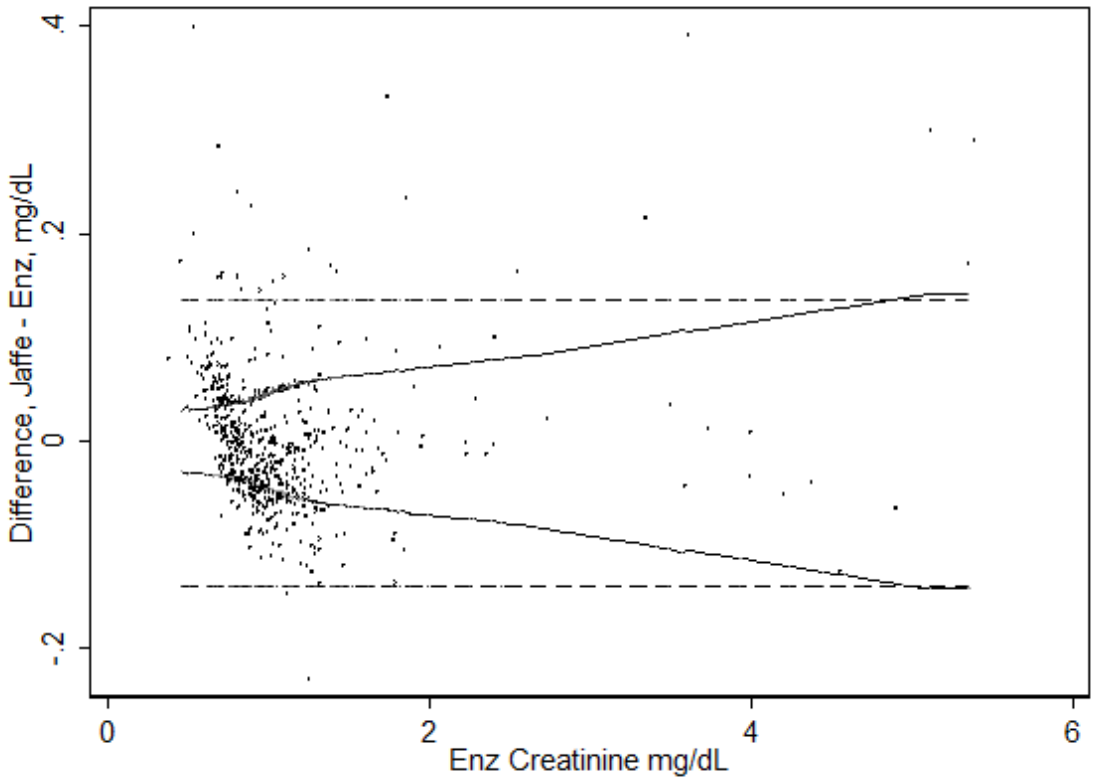

Figure 2: Difference plot for creatinine. The solid lines represent two standard deviations of the difference between Jaffe and enzymatic methods based on measurement error $s_{C,JE}\,(C_E) = \sqrt{s^2_{C,J}(C_E) + s^2_{C,E}(C_E)}$. The dashed lines represent two standard deviations of the distribution of observed differences.

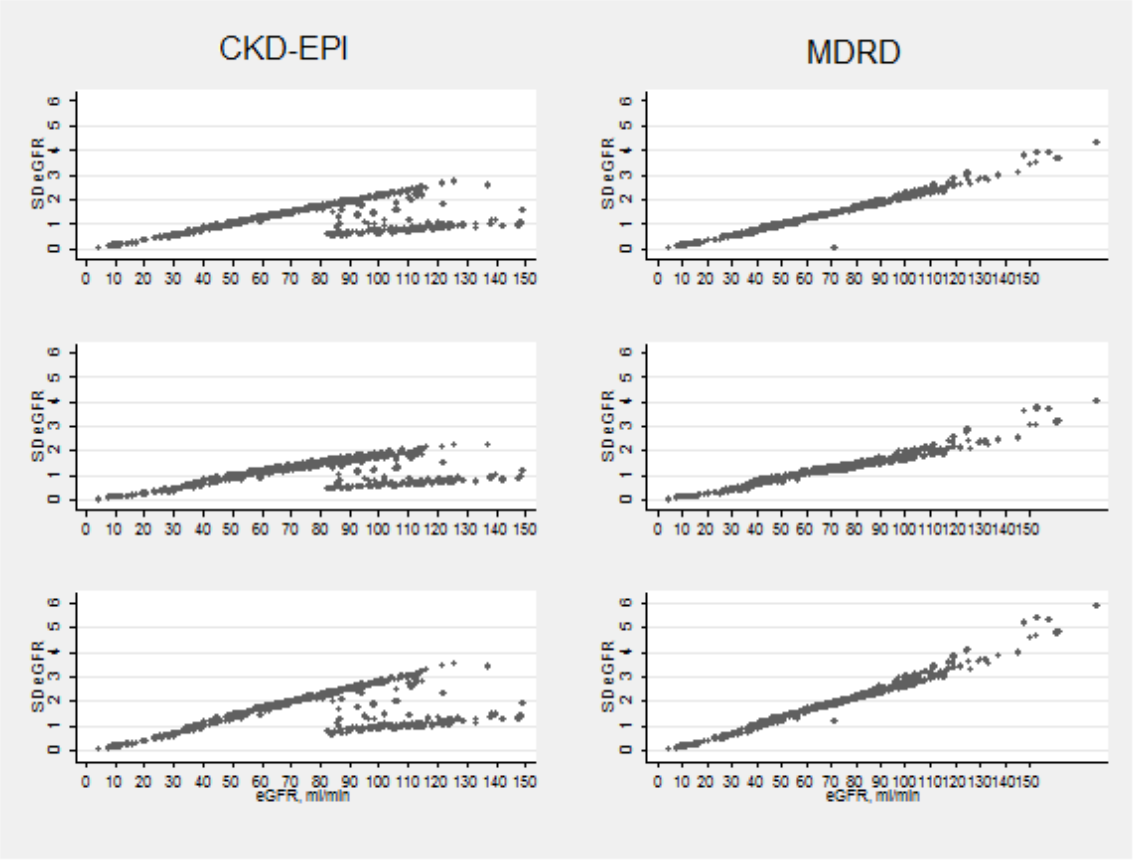

Figure 3: Precision profile for eGFR measurements. The figure shows the standard deviation of the eGFR as a function of eGFR for the CKD-EPI (left panels) and MDRD (right panels) methods. The top, middle, and bottom rows correspond to the enzymatic, Jaffe and error of the difference (Jaffe – enzymatic), respectively. All data (age, sex, and race) are aggregated. The discontinuity in the CKD-EPI plots is due to the maximum and minimum functions in the CKD-EPI equation.

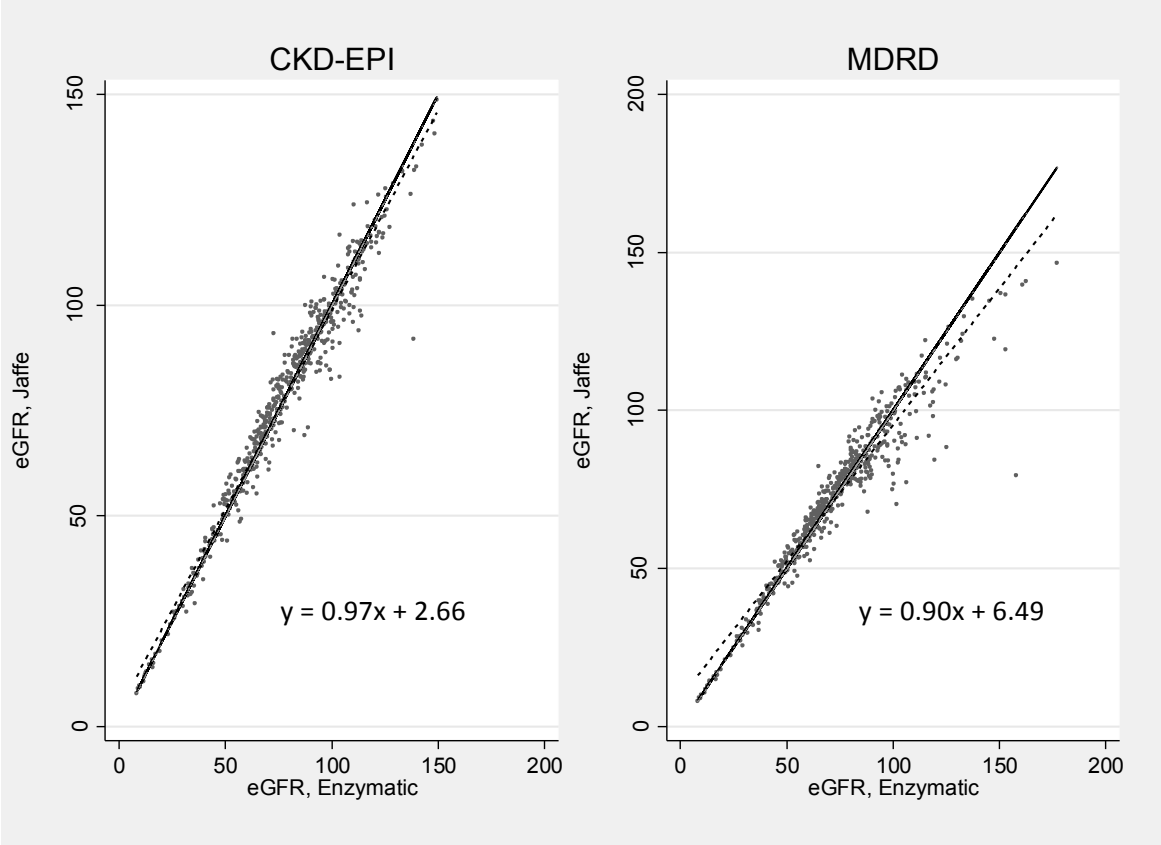

Figure 4: Correlation of Jaffe eGFR and enzymatic eGFR. Left Panel: eGFR based on CDK-EPI equation. Right panel: eGFR based on MDRD equation. Solid line: Line of perfect concordance. Dashed line: Best linear fit.

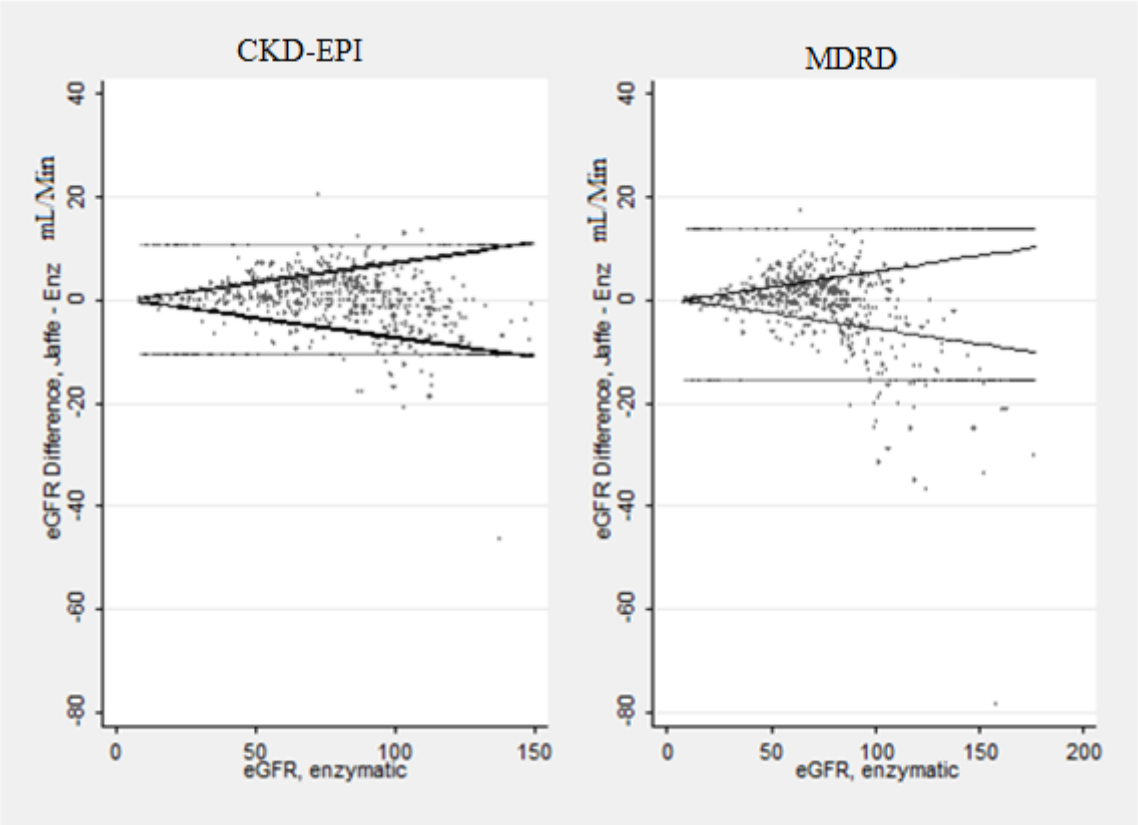

Figure 5: Difference plots for the eGFR by CKD-EPI and MDRD. The outer lines are the Bland-Altman (difference) limits of agreement. The inner lines are the estimated measurement error for the difference of the eGFR for the Jaffe and Enzymatic method.

DISCUSSION

This study examined the differences between serum creatinine results and resultant eGFRs based on the use of enzymatic and Jaffe methods. The study determined the incremental discordances that would arise from the use of the Jaffe method. This study used two different criteria, with different levels of stringency, to define discordant results for: 1) criteria based on observed differences between the Jaffe and enzymatic methods; 2) criteria based on the estimated measurement error of the difference between the Jaffe and enzymatic methods. These criteria were based on potential sources of variation that lead to discordant results.

Multiple factors can create variability in creatinine results. These are categorized as measurement error (imprecision) and nonmeasurement error (sample interferences). Measurement error and nonmeasurement error correspond to common cause and special cause variation in the quality control literature. The goal of this study was to estimate the discordance rate due to interferences in the Jaffe method and to separate out the discordances due to measurement variation.

Unexpectedly, the imprecision of the Jaffe method was lower than the enzymatic method at four of the five concentrations in the 20-day precision profile (Table 4). This is in contrast to the manufacturer precision data provided by Abbott Diagnostics (Table 3). Although the precision data obtained from the UHCL 20-day precision profile was limited to a smaller data size (Table 4, 40 data points per creatinine concentration level

for the UHCL precision compared to 80 data points per concentration level for the Abbott precision), the UHCL precision profile strictly adhered to CLSI precision guidelines to ensure the most accurate results possible. Also, the UHCL precision profile evaluated five total concentrations concurrently with both methods, whereas the Abbott precision data evaluated two concentrations for the Jaffe method and three concentrations for the enzymatic. Neither of these Abbott precision studies evaluated the Jaffe and enzymatic methods concurrently.

The most stringent comparison criterion in this study was based on the estimated measurement error of the difference between the Jaffe and enzymatic eGFRs (Equations 6 and 7). This criterion was based on precision studies for the Jaffe and enzymatic methods. The criterion is stringent because the estimated standard deviation only includes short-term sources of within-run and between-run variation. For example, the criterion used eliminates variation due to different reagent lots, calibrations, instruments, and operators. This produced a lower bound on the standard deviation of the measured differences and an upper bound for the percentage of differences that were due to all nonmeasurement factors. Approximately 40% of the differences between the eGFR based on the Jaffe and enzymatic method could be attributed to nonmeasurement variation. Although this criterion enabled an upper bound on the discordance rate to be set, it is not clear what proportion of such discordances is clinically significant because the threshold for discordance is so low.

The least stringent comparison criterion was based on the observed differences between the eGFRs produced by the Jaffe and enzymatic methods (Equation 5). This criterion included all sources of variation which produced a lower bound for the

discordance rate due to interference. Using this criterion for both the CKD-EPI and MDRD equations, approximately 5% of differences were classified as discordant (Tables 5 and 6).

Figure 6 illustrates the discordances observed at the 60 mL/min/1.73m^2 decision limit for both eGFR calculation methods. A result was classified as discordant if the enzymatic eGFR and Jaffe eGFR classified cases differently with respect to a decision limit. Each line in the graph represents the difference in the Jaffe and enzymatic result of a single sample as it crosses over the 60 mL/min/1.73m^2 decision limit (see Equation 7). Discordances with less than two standard deviations represent normal measurement variation and are marked with circles. Discordances with greater than two standard deviations represent samples with the presence of some form of sample interference and are marked with triangles. The discordances marked with triangles represent significant changes in the eGFR at critical decision limits and may result in a change of care to the detriment of the patient.

The number of discordant results varies by which eGFR calculation is used. Using the CKD-EPI calculation, a total of 17 discordant results are recorded at the 60 mL/min/1.73m^2 decision point. Using the MDRD calculation, a total of 26 discordant results are recorded at the 60 mL/min/1.73m^2 decision point. The magnitude of individual discordances is also greater with the MDRD equation and is visualized in Figure 6. The importance of discordant results at this decision limit is the reclassification of renal function (Table 2) and, more importantly, potential for change in disease treatment.

Comparing the estimated discordance rates using the CKD-EPI and MDRD equations shows a small degree of variability at different eGFR decision limits (Tables 7

and 8). The MDRD equation presented a lower observed discordance rate at the 30 and 45 mL/min/1.73m^2 decision limits. The CKD-EPI equation presented a lower observed discordance at the 60 mL/min/1.73m^2 decision limit, as well as a lower estimated discordance rate due to factors other than imprecision at the 30 and 60 mL/min/1.73m^2 decision limits.

The number of observed discordant eGFR results slightly varies based upon which calculation is used. Using the CKD-EPI equation, 217 of 543 of eGFR results were discordant based upon imprecision, and 21 of 543 of eGFR results were discordant based upon observed differences (Bland-Altman limits of agreement). Using the MDRD equation, 217 of 543 discordant results were observed based upon measurement error and 26 of 543 discordant results were observed based upon observed differences (Bland-Altman). The MDRD equation shows lower correlation (Figure 4) of results and a greater magnitude of variation (Figures 5 and 6) than the CKD-EPI equation.

The question arises, "Are the costs savings worth the risk of implementing the Jaffe methodology?" This study determined that 40% of the differences in eGFR exceeded two standard deviations of the estimated measurement error (most stringent criteria), and only 5% of the differences exceeded two standard deviations based on observed differences (least stringent criteria). Risk is the product of two components: 1) the probability of an event; and 2) the consequence associated with the event. This study has determined the probability of a discrepant creatinine result. Future research to be conducted on this topic is to determine the clinical consequence of a discrepant creatinine result.

This study is the first step in providing cost savings while continually improving

patient care. The potential cost savings for the UHCL switching to the Jaffe method for outpatient and clinic sample testing is tremendous. With the recent implementation of the Affordable Care Act (ACA), and its associated laboratory reimbursement cuts,[28-31] laboratories are being driven to find cost savings. However, changes in the laboratory to provide cost savings should not come at the expense of patient care. Should this method be deemed safe for use, UHCL would achieve substantial savings. Further discussion between laboratory management and physicians is still required to determine if the less expensive Jaffe method is a safe alternative for this outpatient population.

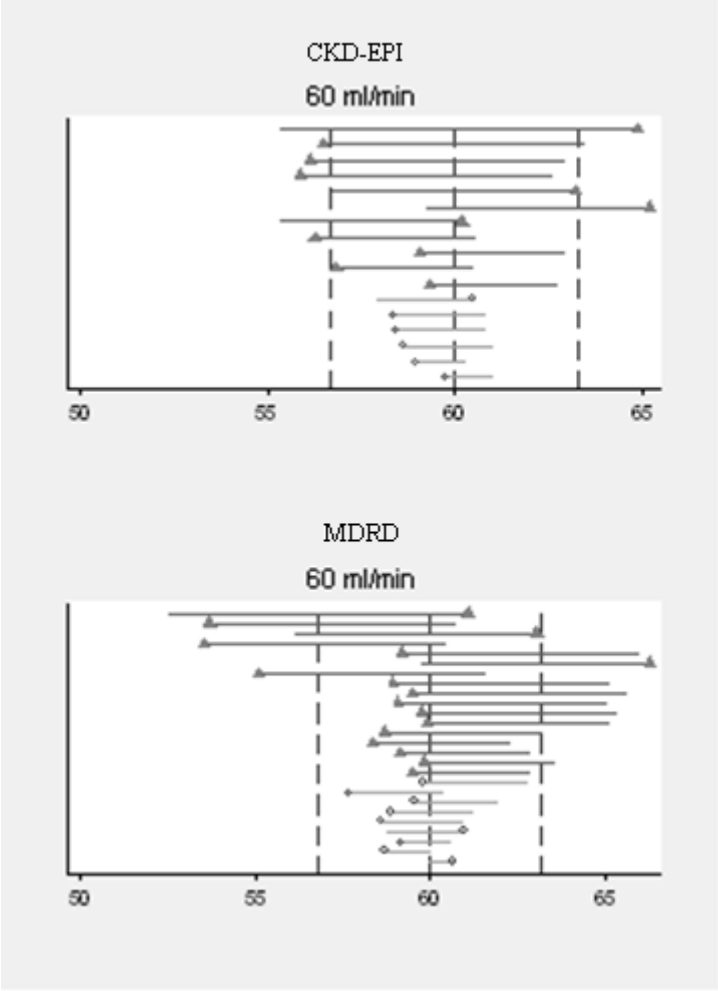

Figure 6: Discordances at decision limits. Each line represents the difference between the Jaffe eGFR and enzymatic eGFR (indicated by marker) of a single patient. The vertical lines indicate the decision limit and two standard deviations of the difference (Jaffe-enzymatic) due to measurement error (Equation 7) at the decision limit. Differences that are greater than two standard deviations of the measurement error are indicated by triangles. Differences less than two standard deviations are indicated by circles.

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